# THE NATURE OF THE POLYHEDRAL BODIES FOUND IN INSECTS.[1]

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CONTENTS.



## INTRODUCTION.

A very large group of lepidopterous larvæ is subject to a class of infectious diseases known as the "polyhedral diseases." Whether the organism concerned in their production is identical or not for all the species of insects affected must remain a matter of conjecture till further work allows us to venture an interpretation. One thing, however, is certain, namely that curious crystal-like structures called "polyhedral bodies" or "polyhedra" are always associated with the type of diseases we are here discussing. Although these polyhedra may vary considerably in size and somewhat in shape in the different species of insects, nevertheless, they are always specific for a certain type of malady.

Wahl, followed by Prowazek and Escherich, consider the polyhedral diseases as distinct and absolutely divorce them from the fungous, protozoan and bacterial affections of insects. We believe that the erection of a separate group to embrace all of the polyhedral diseases is an excellent plan and receives our sympathetic endorsement, for the reason that the confusion of all of the insect diseases is still common amongst entomologists. We venture to say that there are scarcely two entomologists in America who know the difference or similarity between any of the diseases expressed by such terms as Muscardine, Pébrine,

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flacherie, lethargia, maladie de morts-blancs, schlaffsucht, faulsucht, fettsucht, polyhedral disease, wilt, wipfelkrankheit, jaundice and gelbsucht.

We do not wish to dwell upon the differences or similarities between all of these maladies, but will confine ourselves solely to the group of polyhedral diseases under which are included the four commonest manifestations, viz., wilt, wipfelkrankheit, jaundice and gelbsucht. Wilt is the vernacular term used in America for the polyhedral diseases. It is a name suggestive of post-mortem aspects, but unfortunately a number of caterpillar diseases distinct from wilt on superficial examination also have a similar post-mortem appearance. Moreover, a number of diseases common to plant pathology are labelled with the same term. We do not think it wise to eliminate the name, however, for the reason that it has been used so long and "wilt" certainly means more to field entomologists and foresters than "polyhedral disease" which is only significant to laboratory workers. The term "Wipfelkrankheit," which is used for a similar affection of nun moth caterpillars in Germany, is a very suggestive name for the reason that when the animals are in the last stages of the disease, they congregate in masses at the tops of the trees or "wipfeln" and die hanging by their prolegs. The name "Wipfelkrankheit" has the further advantage in that it is not applied to any other plant or animal disease. "Gelbsucht" or "jaundice" are terms used to designate a polyhedral disease in silkworms. The two terms are descriptive of the clinical picture of the diseased worms and are not used for any other affection known to pathology.

The larval stages of the following species of lepidoptera have been examined by us and found to be susceptible to the polyhedral diseases. Animals which can only be infected experimentally have been omitted, *i. e.*, the list comprises only those which besides being capable of experimental infection also have the disease or diseases in a state of nature.

I. Saturnidæ.
   1. *Hemileuca maia* Drur.
II. Arctiidæ.
   2. *Apantesis virgo* Linn.

III. Noctuidæ.
  3. *Leucania unipuncta* Halw.
  4. *Noctua clandestina* Harris.
  5. *Autographa brassicæ* Riley.
IV. Lymantridæ.
  6. *Porthetria dispar* L.
  7. *Lymantria monacha* L.
  8. *Orgyia leucostigma* S. and A.
V. Lasiocampidæ.
  9. *Malacosoma americanum* Fabr.
  10. *Malacosoma disstria* Hubn.
VI. Bombycidæ.
  11. *Bombyx mori* L.
VII. Dioptidæ.
  12. *Phryganidia californica* Packard.
VIII. Pieridæ.
  13. *Colias philodice* Godart.

From a perusal of the above list it will be seen that the polyhedral diseases are very widely distributed and affect some of our most important economic insects. We have estimated that epidemics of polyhedral diseases at certain times kill off from 30 to 70 per cent. of some of our most noxious pests. This is especially true, as we have found in connection with our studies on the gipsy moth, tent caterpillars and army worms. The polyhedral diseases contribute much more to the control of certain of our noxious caterpillars than the combined efforts of all their hymenopterous and dipterous parasites. Therefore, we believe that these diseases merit a serious consideration from all points of view.

## The Polyhedral Bodies.

Caterpillars dead from wilt are usually found on some elevated place hanging by their prolegs. Dead nun moth caterpillars are found hanging from the very highest branches of a conifer, dead gipsy moth caterpillars are found hanging anywhere on the trunk of a tree or on a branch; the favorite dying places of the American tent caterpillars being usually in close proximity to the nest on the branches of an apple or cherry tree. The army

worm seeks the tip of a grass blade and succumbs thereon. An innate desire to reach an elevated place on their favorite food plant always seizes the diseased insects prior to death. We have never observed animals in the last stages of wilt descend their food plants. So far we are unable to offer any explanation which would satisfactorily assist in analyzing this ascending instinct. A short time after death, the animals become deliquescent. At the slightest touch the skin ruptures and a dark brown liquid oozes out. In some species such as the American and forest tent caterpillars this liquid is pink shortly after death and becomes dark brown later. The corpses will be practically odorless if they have hung but a short time and before septic bacteria have gained a foothold.

If some of the brown liquid from a dead caterpillar is examined microscopically with a high-power dry or oil-immersion lens, it will be found to contain, besides the elements of disorganized tissues, myriads of polyhedral bodies of various sizes. (Fig. 1, Plate 1.) Certain polyhedra have been found to measure $\frac{1}{2}\,\mu$ and less in diameter while still others reach the size of $15\,\mu$. The average polyhedron of the gipsy moth caterpillar measures $3.4\,\mu$ in diameter. The bodies in this species are larger than those in any other form we have hitherto examined. The average size of nun moth caterpillar polyhedra measure $2.65\,\mu$ in diameter; those of the forest tent caterpillar $2.6\,\mu$ and so on until we come to *Phyrganidia californica* and the tussock moth caterpillars in which the average diameter has been found to be $1.6\,\mu$ and $1.5\,\mu$ respectively. Thus it is seen that the average polyhedron varies greatly in size in the different species. As stated previously the sizes of the polyhedra within one species or even within one animal (gipsy moth: $\frac{1}{2}\,\mu$–$15\,\mu$) varies also. There exists a striking similarity between the shapes of these bodies in the different species but some variation within a particular species or even within the same animal can be observed. In general the form is that of a polyhedron with more or less rounded angles. They never assume the shape of a perfect sphere, and an actual geometric outline has never been observed except in the silk worms where almost perfect octahedra are found. The polyhedra are highly refractive, and on focusing are seen to have a

denser center differentiated from a somewhat lighter periphery. Sometimes within the bodies concentric layers like those of an onion are observable. Often two polyhedra are seen adhering to one another as if in the act of dividing, but an actual division in a hanging-drop has never been observed. When pressure is applied to the cover glass, the polyhedra crack very readily into a number of pieces, and often without the application of pressure the same fragmentation may be observed to occur somewhat more slowly. In the latter case a notch appears at one side of the polyhedron which gradually lengthens into a line progressing slowly toward the other side, much like the cracking of ice. Usually before the line has completely separated the two halves other lines appear, and soon the entire polyhedron is divided into a number of pieces, which may separate or may stick together in a rosette-like fashion. At no time was anything observed to come out of the polyhedra when they cracked in this manner. If the cover glass is moved while applying a little pressure, one half of the polyhedron may sometimes be folded upon the other half without the cracks appearing, showing that it is composed of a tough substance and is not at all brittle like inorganic crystals.

The only objects in a fresh preparation with which one could possibly confuse the polyhedra are the fat globules and urate crystals, but with a little practice these may be readily distinguished. Fat globules are perfectly spherical and are therefore unlike the polyhedral shape of the bodies in question; but when in doubt, Sudan III was used, for in this stain the fat globules become red, while the polyhedra remain colorless. The urate crystals are often more acutely angular or are of an entirely different shape from the polyhedra and are frequently traversed by radiating lines.

Besides polyhedral bodies, fat globules, and urates, a smear from a newly "wilted" caterpillar contains cellular débris, hairs and pigment granules. The pigment granules must not be confused with bacteria, for many of them superficially resemble these organisms very closely. When a preparation is dried, mounted, and examined under oil, the pigment granules of the gipsy moth may easily be confused with small micrococci, owing to the fact that they are usually arranged in pairs. As a

matter of fact, a smear made from a recently wilted caterpillar is almost devoid of bacteria, and in many cases none at all can be found. If bacteria are present, they have escaped into the body cavity through rupture of the intestine and bear no direct etiological relation to the disease.

In fixed and stained smears a number of things can be demonstrated to advantage within the polyhedra. Fixation was accomplished either by passing the preparation through a flame or by placing it in absolute alcohol for a few minutes. The smears were then stained in Giemsa's solution for 12 hours or were stained for a shorter period with one of the following dyes: Methylene blue, trypan blue, gentian violet, carbol fuchsin, Bismarck brown, or iron hæmatoxylin. When iron hæmatoxylin was used, the preparation was first mordanted in a 4 per cent. ferric-alum solution for two or three hours. After staining, the preparations were sometimes quickly passed through the alcohols to xylol before mounting. This not only clears everything, but dissolves away all the fat on the slide and thus increases the transparency of the preparation. Gipsy moth polyhedra are rather resistant to stains in general and usually color along the periphery only, unless the stain is applied for a long time. When this is done one may succeed in staining the entire polyhedron, especially after the use of some mordant like ferric alum before hematoxylin or anilin water before gentian violet. Steaming the preparation with a stain like carbol fuchsin has also given good results. When properly stained, one of three conditions is observed: First, the polyhedral bodies are uniformly stained so that nothing can be detected within them; or second, a uniformly darker staining central mass can easily be differentiated from an almost unstained outer substance; or, third, many little refractive, reddish granules are seen within the polyhedra. An actual differentiation between what might be interpreted as nuclear and cytoplasmic material within the polyhedra never occurs. Therefore, in accounting for the staining reactions we believe that at times the polyhedra have a central granular or homogeneous substance easily distinguishable from an outer tougher substance which is more resistant to the dyes. This varies a great deal, however, and sometimes the periphery takes the

stain more readily than the underlying strata. From these staining reactions it becomes apparent that the polyhedra are complicated in structure, and do not therefore differ essentially from what Bolle and Prowazek found to be true of the silkworm polyhedra. Our observations on the staining reactions also show that morphological studies do not enable us to regard the polyhedral bodies as organisms. We believe that the polyhedra are protein degeneration-products of the disease. The staining reactions have demonstrated that they are not simple crystals, but complicated in structure, and have a tough outer layer. Consequently, and for a number of other reasons, we do not believe them to be true crystals and therefore choose to call them pseudo-crystals. The variations in their staining reactions which one obtains at times can well be accounted for by assuming that one is dealing with different stages in the synthetic process of pseudo-crystals. Another matter militating against the idea that the polyhedra are organisms is the fact that Glaser ('15) and Chapman and Glaser ('16) have experimentally demonstrated the possibility of infecting healthy gipsy moth caterpillars with wilt material from which the polyhedral bodies were removed by passing the virus through Berkefeld Grade "N" candles. We have shown that wilt is caused by a filterable virus and believe that the polyhedra arise as a reaction against the invasion of this virus.

## Origin of the Polyhedral Bodies.

Studies on sectioned gipsy moth, army worm and tent caterpillar material have shown that the polyhedra originate within the nuclei of the hypodermal, fat, tracheal matrix, and blood cells. (Fig. 2.) In the true army worm, however, polyhedra are at times formed within the nuclei of intestinal epithelial cells. We have been utterly unable to find the bodies within the nuclei of muscle tissue, Malpighian tubes, ganglia, nerves, œnocytes, salivary glands, gonads and within the intestinal epithelial cells of all forms except the true army worm.

The formation of the polyhedral bodies within the nuclei of the four tissues above mentioned and the visible changes taking place within these nuclei may be described as follows: The first

indication of a diseased nucleus seems to consist in the flowing together of the chromatin into a lump in the middle. Then out of the achromatic substance the polyhedra arise as very minute individuals. (Fig. 2, Plate I.) They gradually increase in size and probably most of the chromatin is also used up during the synthetic process. As the polyhedra grow, they become more and more refractive, stain with difficulty, and the nucleus becomes hypertrophied. The late stages of these hypertrophied nuclei are more than twice as large as the largest normal nucleus. (Fig. 2, Plate 1, and 3, 4 and 5, Plate II.) This swelling of the nucleus is due to the increase in size of the polyhedral bodies which stretch the nuclear membrane. During the earlier stages of the disease the polyhedra are somewhat rounder than the larger ones found later on prior to death. This can be accounted for by the fact that, as the polyhedra grow, they become so closely packed within a nucleus that they press upon one another and thus the more or less polygonal shape is produced. As the polyhedra grow and become more refractive the remains of the chromatin lump disappears and there remains simply the nuclear membrane enclosing the polyhedra. (Fig. 2, Plate 1, and 3, Plate II.) Finally the nucleus disintegrates, and the polyhedra are found free in great numbers in smears of dead caterpillars. (Fig. 6, Plate II.) We believe (and this belief is based on morphological, chemical and experimental evidence) that the polyhedral bodies are degeneration-products of the disease—products of nuclear disintegration. This view may not seem so improbable if one reviews some of the literature dealing with a few of the diseases in higher animals.

### Hydrophobia (Rabies).

In 1903, Negri described certain bodies occurring in the nervous system of animals dying of rabies. The bodies seem to be specific to the disease, and are of great assistance for diagnostic purposes. The Negri bodies vary in size, measuring from .5 to 25 $\mu$. They are round, oval or angular in outline and are found in the protoplasm of the nerve cells and their processes. The bodies occur in all parts of the nervous system, but are most common in the Purkinje cells of the cerebellum and especially in the cells of the cornu Ammonis. The virus of hydrophobia passes through

the coarser Berkefeld and Chamberland filters, and these filters exclude the Negri bodies. The most generally accepted view at present is that the Negri body is a cellular reaction against the invasion of the filterable virus. The virus of hydrophobia has not been cultivated.

### Variola and Vaccinia (Smallpox and Cowpox).

In 1892 Guarnieri described certain cellular inclusions in variola and vaccinia which are specific for these two diseases. The bodies are from 1–8 $\mu$ in diameter, and round, oval, or sickle-shaped. They lie in the cellular spaces, often in close proximity to the nucleus, and can be demonstrated in vaccine pustules as well as in the experimental lesions produced in the rabbit's cornea. The virus of variola and vaccinia passes through the coarser porcelain (Chamberland) filters. Most authorities regard the Guarnieri bodies as the effect of a specific reaction of epithelial cells against the virus. The virus has not been cultivated.

### Trachoma.

In trachoma certain granules are found in the cytoplasm of the inflamed epithelial cells which cover the conjunctiva. Bodies similar to the trachoma bodies have also been found in other inflammations of the conjunctiva, and are therefore thought not to be specific of trachoma. The trachoma bodies are regarded as the product of mucous secretion under pathological conditions. The virus passes through Berkefeld filters. It has not been cultivated.

Cellular inclusions not regarded as parasitic by some workers have been found in a number of other diseases. In scarlet fever cellular inclusions occur in the skin lesons; in foot and mouth disease inclusions are found in the vesicles; in fowl pest inclusions are found in the brain and in epithelioma contagiosum or fowl diphtheria in the epithelium. Cytoplasmic inclusions accompanying many of the diseases of higher animals, and regarded as non-parasitic in most cases are not at all uncommon, but we have been unable to find any account in the literature of vertebrate diseases which are accompanied by the formation of *nuclear* inclusions as is the case with the polyhedral diseases of insects.

## Bio-chemical Observations on the Polyhedral Bodies.

According to Tubeuf, Krassilschtschik, Prowazek, Wahl, Wolff, Glaser and Chapman the polyhedral bodies are regarded as being reaction products; towards bacteria (Tubeuf, Krassilschtschik) or towards Chlamydozoa (Prowazek, Wolff) or towards an unknown virus (Wahl) or towards a filterable virus (Glaser and Chapman). According to Bolle, Fischer, Marzocchi, and Knoche the polyhedral bodies are stages of a protozoan; according to Escherich and Miyajima they are bearers of an unknown virus.

It seems that the views are very diverse as regards the true nature of the polyhedra. For this reason it was thought advisable to submit some of our bio-chemical work on this subject in further support of our contention, obtained from morphological and experimental studies, that the polyhedra are merely organic degeneration-products of the disease.

During the gipsy moth season great quantities of diseased material can be obtained. For this reason all of the bio-chemical investigations were performed with gipsy moth polyhedra. These bodies are heavier than water and consequently can be obtained in bulk by centrifuging aqueous emulsions of diseased material. By repeated washing, filtering and centrifuging most of the fat, cellular débris, etc., can be eliminated. After this treatment the polyhedra were always washed with ether in order to free them from any possible remnants of adhering fat. Thorough attention to this cleansing operation will yield polyhedra in a fairly pure state for chemical tests. The material was allowed to dry naturally in the centrifuge tube, after which the lump that formed at the bottom could be loosened and transferred to a mortar where it was pulverized. This pulverization if done gently does not crack or injure the polyhedra in any way. After this procedure the mass of polyhedra look very much like pulverized chalk. It is comparatively easy to obtain 2 or 3 grams of polyhedra from about one or two hundred caterpillar cadavers.

As the polyhedra do not blacken with osmic acid, and do not stain with Sudan III., it seems unlikely that they contain fat. They stain with picric acid, however, and this gave us the clue to their possible protein nature. The color tests for dry proteins

were then applied and we obtained positive reactions with xanthoproteic, Millon's, biuret, Adamkiewicz's and Lieberman's tests. It was next found necessary to obtain the polyhedra in solution so that the various coagulation or precipitation tests could be performed.

Before testing the solubility of the polyhedra in various reagents they were first rubbed energetically in an agate mortar with the addition of a little sea sand. This grinding was found necessary for the reason that the outer surface of the bodies is composed of more resistant material than the underlying strata. By grinding with sea sand the polyhedra are fragmented and this offers more delicate surfaces to the action of the reagents.

The polyhedra were found to be insoluble in hot or cold water, alcohol, chloroform, ether, or xylol. They dissolve readily in strong acids and alkalies, but these reagents were thought to produce too great a hydrolytic cleavage of the protein molecule, and since we did not wish to alter our material to any appreciable extent a number of milder reagents were tried. Moreover, from the standpoint of the classification of proteins it is important to determine just what will and what will not dissolve the material.

The following solubility tests were performed. Two grams of ground polyhedra were divided into four parts. To ½ gram water was added; to ½ gram .5 per cent. and to another ½ gram 2 per cent. NaCl solution, and to the fourth ½ gram 10 per cent. $Na_2CO_3$ were added. The tests were kept over a water bath for 13½ hours at a temperature varying between 55° and 58° C. At the end of this time the solutions were filtered and the various tests for soluble proteins applied. All of the tests (acetic acid, nitric acid, cupric sulphate, mercuric chloride, acetic acid with potassium ferrocyanide and ammonium sulphate) were negative showing that nothing went into solution.

Two grams of polyhedral material were again divided into four parts and treated respectively with $H_2O$, .5 per cent. and 2 per cent. NaCl and 10 per cent. $Na_2CO_3$. The tests were placed over a small direct flame for two hours. At the end of this time the solutions were filtered and the tests for soluble proteins applied. Negative tests were obtained with the water and salt

solutions. The material treated with the 10 per cent. $Na_2CO_3$ solution gave slight coagulation tests with acetic and nitric acids showing that a small amount of protein went into solution. The polyhedra treated with the carbonate were examined microscopically and it was found that some fragments had partially dissolved while most of the polyhedra, which had apparently resisted fragmentation in the mortar had swollen to double their normal size.

Concentrated HCl (37 per cent.) used both hot and cold seems to dissolve the polyhedra with difficulty. The solubility of the bodies in boiling $HNO_3$ seems to lie between 15 and 20 per cent. We began with 4 per cent. $HNO_3$ in which the polyhedra are not affected and worked up to 31 per cent. $HNO_3$ in which they dissolve instantaneously on boiling. The fact that the liquid clears when some of the lower percentages of $HNO_3$ are used is not sufficient evidence that all of the polyhedra have been dissolved. For this reason the solubility of the bodies towards the acid was checked by microscopical examinations.

$(NH)_4OH$ does not seem to affect the polyhedra, but the other alkalies such as KOH and NaOH dissolve them readily. It was found that as low a percentage as 1/16 per cent. NaOH will dissolve polyhedra if they are boiled in the solution. For convenience 2 per cent. NaOH was used for the following tests. Two grams of ground bodies were dissolved in the alkali by means of heat. The solution was then dialyzed in order to get rid of the alkali. The dialysis was usually complete after 24 to 48 hours. At the end of this procedure the proteins remain in solution (*i. e.*, on dissolving in alkali, after which, although the alkali be removed, the polyhedra proteins remain soluble) and the tests for soluble proteins can be applied. We obtained positive reactions with acetic acid, nitric acid, cupric sulphate, mercuric chloride, acetic acid with potassium ferrocyanide and ammonium sulphate.

It might be well to mention that the percentages of NaOH used were accurate and the material pure. In making up the solution we did not rely on the so-called purity of the hydroxide sticks, but always eliminated every trace of $Na_2CO_3$ by precipitation with $Ba(OH)_2$.

After determining that the polyhedra are protein in nature it

was next thought advisable to ascertain whether or not they are nucleoproteins. It seemed likely that this would be the case for the reason that they are formed in the nuclei of certain tissue cells. Two grams of whole (unrubbed) polyhedra were digested for one week in artificial gastric juice. After this time a microscopic examination failed to reveal any polyhedra. Theoretically, everything should have been decomposed excepting the nucleins which have a high phosphorus content. At the end of one week's digestion the material was filtered through and dried on ash free filter paper. The paper containing the residue was then cut into fine pieces and put into a platinum crucible with an oxidizing mixture ($Na_2CO_3$ (2 parts) + $KNO_3$ (1 part)). The material was slowly ignited and the residue dissolved in weak $HNO_3$. This solution was then warmed with the addition of some $NH_4NO_3$ in order to make the expected precipitate less soluble. Lastly 5 per cent. molybdic acid was added. The material was placed in an incubator and on standing a pronounced yellow precipitate (ammonium-phospho molybdate) was formed.

On the basis of this and the other tests described the polyhedra meet all of the requirements of the nucleoproteins. Nucleoproteins give all the color reactions, are soluble in water containing a small amount of alkali (1/16 per cent. in case of polyhedra) and are precipitated from this solution by acetic acid. The nucleins which have a high phosphorus content are not decomposed by gastric juice, and are obtained as an insoluble residue after the artificial digestion of nucleoproteins with pepsin.

Since iron is an element known to be contained in chromatin it was further thought advisable to determine whether the polyhedra during their synthesis from the chromatin and other substances in the nuclei embodied any iron. It will be needless to go through all the details of the analysis. Suffice it to say that every precaution to prevent iron contamination from water, air, etc., was used. Reagents known to be free from iron contamination were employed. Furthermore, the polyhedra were not rubbed with sand for fear of introducing iron in this manner. .0988 of a gram of polyhedra were used and .00049 of a gram of iron was found.

On dissolving polyhedra in alkali and after dialyzing away

the alkali, three fractional precipitations with magnesium sulphate or sodium chloride can be obtained. Whether this means that the polyhedra are composed of three separate proteins or three groups of proteins, we are not prepared to say. The three precipitates, however, do demonstrate that the polyhedra are complex and not at all simple, a fact which does not seem strange when one reflects on the complexity of cellular or nuclear material in general.

As stated previously we regard the polyhedra as degeneration products formed during the course of the disease in the nuclei of certain tissue cells. The bodies are nucleoprotein crystal-like (pseudo-crystalline) aggregates. The idea suggested itself to us that it might be possible to dissolve the polyhedra and recrystallize them again after dissolution. If this should prove to be possible, it would militate seriously against the views held by Bolle, Fischer, Marzocchi, Knoche, Escherich and Miyajima that the bodies are organisms or the stages of an organism. Our experimental attempts at recrystallizing the polyhedra are a bit varied and so we do not wish to overemphasize our results as yet, but submit them with a full appreciation of their preliminary value.

Polyhedra were dissolved in 2 per cent. NaOH by heating. The process of dissolution was followed by an examination of samples microscopically, and when traces of the bodies could no longer be observed, the material was evaporated over a water bath and examined before it became entirely dry. Besides long $Na_2CO_3$ crystals we found many small and large bodies which resembled polyhedra. As a check we evaporated ordinary 2 per cent. NaOH, but we could not find the polyhedra-like crystals obtained with the protein solution. This seemed to be a result which offered possibilities, so we proceeded more carefully with another experiment.

Two grams of polyhedra were dissolved by heating in 2 per cent. NaOH. This solution was filtered and washed with ether in order to rid the material of any traces of fat. The ether was then eliminated by means of a separating funnel. The solution was next dialyzed to get rid of the alkali and a few protein tests were performed with some of the solution just to convince our-

selves of the presence of proteins. This protein solution freed from the alkali was now slowly evaporated. On partial evaporation, we found beautiful single and double crystals which simulated the polyhedra very closely. If the material is evaporated completely it is difficult to find the crystals owing to the presence of coagulated and other protein material which hides them. If one shoots water under the cover-slip, however, the crystals again become visible just as soon as the coagulated sediment softens and becomes transparent. This fact that a coagulated residue remains shows that the entire protein material contained in the original polyhedra is not used during the formation of these new crystals. The majority of the crystals produced in this way simulate polyhedra very closely, but some are rounder and larger. Double forms are very common and are absolutely indistinguishable microscopically from ordinary polyhedra. The staining reactions of the crystals are similar to those of the polyhedra and Millon's reaction is identical. We have as yet not obtained a sufficient amount of these new crystals to submit them to all of the protein tests applied to the polyhedra. The crystals are not quite as stable as the polyhedra. They seem to lack the more resistant outer layer and therefore are more easily soluble in alkali and other reagents. For this and other reasons we do not claim to have reproduced typical polyhedra after their disintegration, but we firmly believe that the results are suggestive. It seems unreasonable, after submitting proteins to the violent hydrolytic action of both heat and alkali, to expect to reproduce the identical proteins. However, in the material under consideration, there seems to be a tendency for this particular protein or group of proteins to crystallize out in the shape characteristic of the polyhedra. These observations seem to support our view that the polyhedra are merely degeneration-products and not some inexplicable, unclassifiable organisms as supposed by many workers. An organism certainly could not be dissolved and its original form again reproduced or very nearly reproduced on evaporation.

So far we are unable to obtain the crystals after the dissolution of the polyhedra at every trial. Out of possibly ten trials, one usually succeeds four or five times. Undoubtedly some condition

of which we are at present ignorant is responsible for the frequent failures. We have performed a sufficient number (15) of these recrystallization experiments, however, to warrant a report of the results.

Crystallizable proteins are, of course, not uncommon. Hæmoglobin is perhaps the best known example in animals and the aleurin grains a well known example in plants. By fractional precipitation with magnesium sulphate or sodium chloride two or three separate crystalline proteins can be obtained from the albumen of the hen's egg. In insects, by the evaporation of blood with a trace of acetic acid, beautiful protein crystals can be obtained, different in every species.

Our view regarding the nature of the polyhedral bodies may therefore be summarized as follows: During the course of the disease the virus disintegrates the nuclear material in such a way that crystal-like bodies called polyhedral bodies or polyhedra are synthesized out of the disintegrating proteins. Just how the process from nuclear material to polyhedra takes place is at present unknown. At any rate, from our morphological observations, experimental infection data (published elsewhere) and from our chemical studies here presented, it seems clear that the polyhedra are nucleoprotein degeneration-products and not organisms responsible for a series of insect diseases.

## Summary.

1. Polyhedral bodies are found in many different species of lepidopterous larvæ.

2. The bodies are specific for a certain type of disease.

3. The polyhedra vary in size in the different species.

4. There exists a striking similarity in shape between the polyhedra found in different species.

5. The polyhedra are structurally complicated.

6. They arise in the nuclei of certain tissue cells.

7. Cytoplasmic inclusions are found in certain diseases of higher animals.

8. Nuclear inclusions have not been known previously.

9. The polyhedra are nucleoprotein crystal-like degeneration-products and not organisms.

10. The polyhedra contain iron and phosphorus.

11. On dissolving polyhedra in alkali and after dialyzing away the alkali and evaporating the protein solution crystals are obtained which simulate the original polyhedra.

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PLATE I.

FIG. 1. Photomicrograph of a smear showing free polyhedra.

FIG. 2. Photomicrograph showing various stages during the formation of polyhedra in tissue nuclei of a gipsy moth caterpillar. × 720.

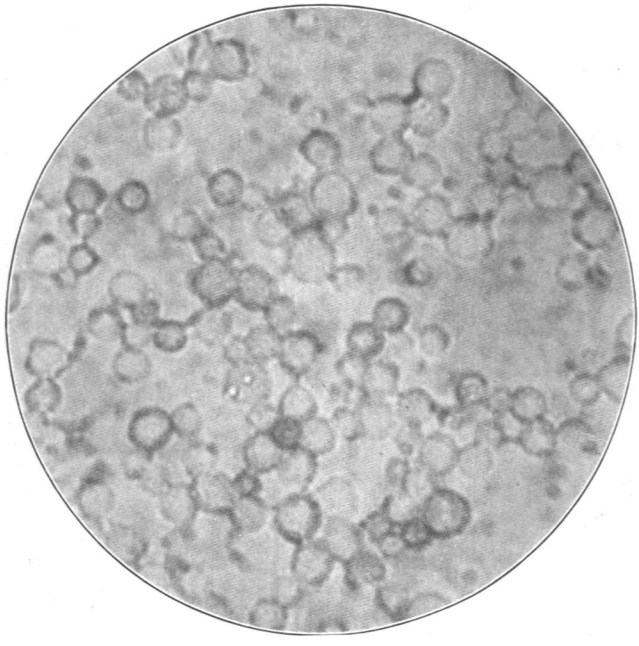

FIG. 1.

FIG. 2.

PLATE II.

FIG. 3. Photomicrograph of army worm tissue showing fully developed polyhedra in nuclei of fat cells. × 300.

FIG. 4. Photomicrograph of army worm tissue showing normal hypodermal, fat, muscle and blood cells. × 300.

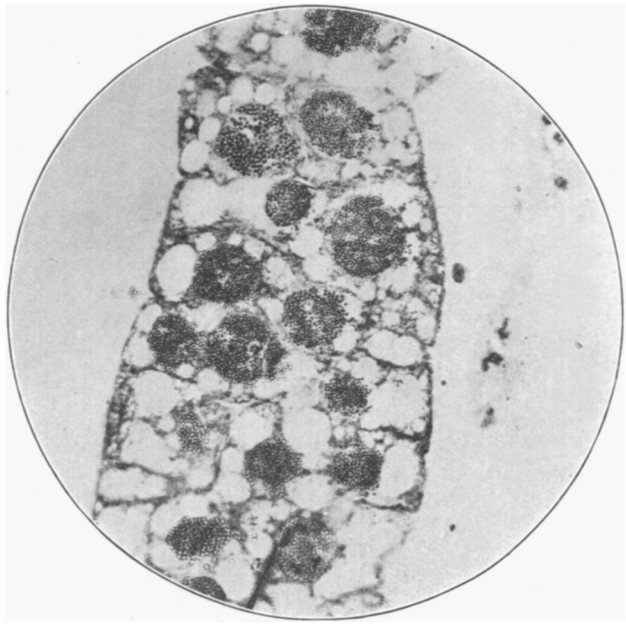

FIG. 3.

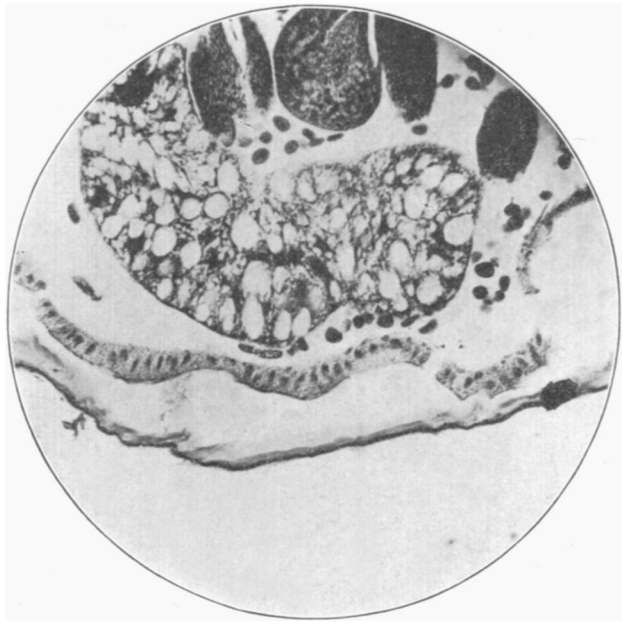

FIG. 4.

PLATE III.

FIG. 5. Photomicrograph of army worm tissue showing normal, hypodermal, fat, muscle and blood cells. × 300.

FIG. 6. Photomicrograph of army worm tissue showing disintegration of nuclei and cells with liberation of polyhedra. × 300.

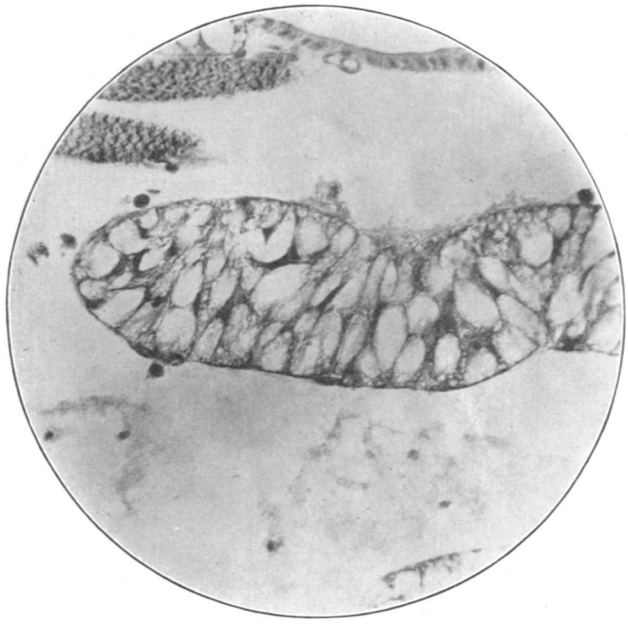

FIG. 5.

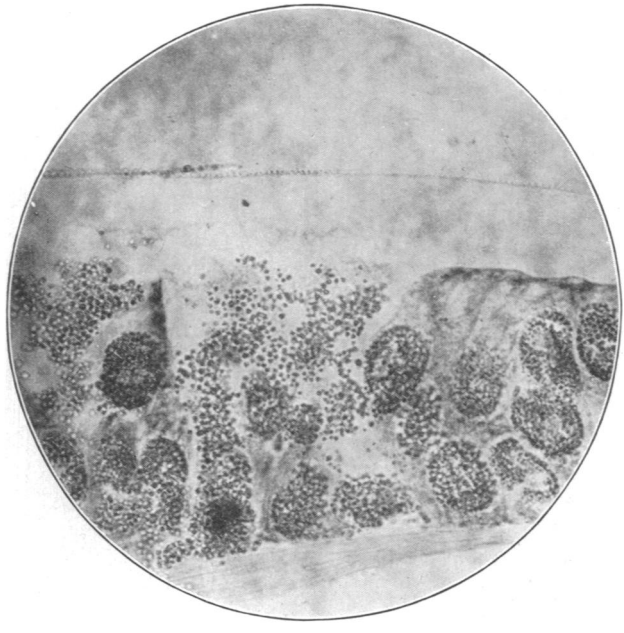

FIG. 6.